ROCK POOL MOSQUITO ECOLOGY OF THE SOUTHERN APPALACHIAN MOUNTAINS

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By

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ABSTRACT

ROCK POOL MOSQUITO ECOLOGY OF THE SOUTHERN APPALACHIAN MOUNTAINS

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The North American rock pool mosquito, *Aedes atropalpus* (Coquillet) (Diptera: Culicidae), is primarily a non-biting species of no perceived threat to public health. The species uses riverine rock pools for immature development and coinhabits the pools with an invasive disease vector, *Aedes japonicus japonicu*s (Theobald) in the United States (U.S.). Since the establishment of the invasive species in the United States in the 1990's, several reports of reductions in *Ae. atropalpus* abundance have led to the hypothesis that the native species is being displaced by the invasive one. The rock pool system remains largely undescribed, limiting our overall understanding of ecological interactions between mosquito species in the system. Here we conducted two studies with the unified objective of improving our fundamental knowledge of rock pool ecology. First, we conducted a field study to describe rock pool communities and analyze the seasonality of rock pool mosquitoes. *Aedes j. japonicus* was present in rock pools at both sites year-round, with overwintering larvae collected in January and winter hatchlings observed in February and March. Early season hatching of *Ae. j. japonicus* allowed the presence

of late instar larvae in pools when the first *Ae. atropalpus* eggs hatched for the season, creating potential for stage-dependent competition between the two species. Such asymmetric competition may be an important factor in the reduction of *Ae. atropalpus* populations. We also conducted a laboratory study aimed at understanding the impact of developmental temperature on *Ae. atropalpus* fitness. We measured common fitness correlates to predict the finite population growth rate for the species at three ecologically relevant temperature ranges. The results illustrate that the fitness of the species suffers at relatively cold temperatures where *Ae. j. japonicus* is commonly found in high relative abundances, but also that the optimal developmental temperature for the native species may be close to that of *Ae. j. japonicus*. The combined results of these laboratory and field studies reinforce prior observations of the importance of temperature in the invasion ecology of *Ae. j. japonicus* and reveal novel observations that will inform further study of the system.

INTRODUCTION

Rock pools are natural containers that support complex communities of vertebrates and invertebrates (Jocque et al. 2010), and the manageable sizes of these contained aquatic environments present ecologists with prime opportunities for field studies of metacommunities (Brendonck et al. 2015; Schiesari et al. 2018). Native and invasive mosquito species are among the diverse assemblages of invertebrates that use rock pools for immature development (Jocque et al. 2010; Byrd et al. 2019). In the United States (U.S.), a common rock pool species is the invasive mosquito *Aedes japonicus japonicus* (Diptera: Culicidae), a potential vector of endemic pathogens including West Nile virus and La Crosse virus (Turell et al. 2001; Sardelis et al. 2002; Schaffner et al. 2009; Turell et al. 2013; Harris et al. 2015). Because of its potential public health importance, research efforts have sought to advance the understanding of this invasive species' life history, ecology, and capacity to vector diseases (Armistead et al. 2012; Hardstone et al. 2012; Kaufman and Fonseca 2014; Reuss et al. 2018), but relatively few studies have primarily focused on its role within rock pool systems. Observational studies have provided evidence that the establishment of *Ae. j. japonicus* in the U.S. has coincided with a reduction in a native rock pool species, *Ae. atropalpus*, in rock pools and other containers (Andreadis et al. 2001; Scott et al. 2001; Bevins 2007; Andreadis and Wolfe 2010). *Aedes atropalpus* is a facultatively autogenous species of no recognized public health relevance (Shaw and Maisey 1956; O'Meara and Krasnick 1970; Telang and Wells 2004; Scholte et al. 2009), and its potential displacement by *Ae. j. japonicus* presents a scenario where an invasive vector of endemic pathogens is replacing a native non-vector.

Understanding the impacts of invasive mosquitoes on populations of native and established species is challenging (Juliano and Lounibos 2005). Invasive mosquito species have been known to negatively impact populations of established species, as evidenced by the decline of *Ae. aegypti* in the United States following the invasion of *Ae. albopictus* (O'Meara et al. 1995). Contrarily, native species can also act as barriers to invasion as demonstrated by the inability of invasive *Ae. albopictus* to occupy bromeliad axils in regions of Florida where native *Wyeomia* spp. use the water-holding plants for larval development (O'Meara et al. 1995b). While *Ae. atropalpus* abundances have reduced following the establishment of *Ae. j. japonicus* in the U.S., it is still abundant in rock pools with temperatures exceeding 30 ^{o}C (Andreadis and Wolfe 2010; Byrd et al. 2019). *Aedes j. japonicus* enjoys the most developmental success in relatively cold water, which may limit the invasive range of the species (Kaufman and Fonseca 2014; Reuss et al. 2018; Montarsi et al. 2019), although the species has become established in subtropical U.S. climates (Riles et al. 2017). It may be possible that *Ae. atropalpus* acts as a competitive barrier to the invasive species at warm temperatures, but is vulnerable to displacement in colder habitats.

Competitive and environmental stress in the larval environment can negatively impact mosquito fitness by increasing susceptibility to arboviral infections (Grimstad and Walker 1991; Alto et al. 2008; Muturi et al. 2011; Bara and Muturi 2015), reducing adult longevity (Reiskind and Lounibos 2009; Alto 2011), lengthening larval development time, and reducing adult body size (Grimstad and Walker 1991; Reiskind and Lounibos 2009; Alto 2011; Costanzo et al. 2011). The native rock pool mosquito has a propensity to reproduce autogenously (O'Meara and Krasnick 1970), a behavior that is directly tied to the generation of teneral reserves through consumption of excessive quantities of nutritive resources in the larval stage (Telang and Wells

2004). This reliance on the acquisition of excessive resources in the larval stage may make *Ae. atropalpus* especially vulnerable to negative effects of environmental stress and competition during larval development (Armistead, Nishimura, et al. 2008a), which could be a factor in the species' reduction in the U.S. following the introduction of *Ae. j. japonicus*. However, competition experiments between the two species have not provided conclusive evidence that *Ae. j. japonicus* is a superior larval competitor (Armistead, Nishimura, et al. 2008a; Hardstone and Andreadis 2012). Those studies used traditional methods like synchronous hatching and static temperatures that are probably misrepresentative of nature, as *Ae. j. japonicus* is known to be a temperate species and an early colonizer of larval habitats (Bartlett-Healy et al. 2012; Kaufman and Fonseca 2014; Cunze et al. 2016; Reuss et al. 2018) while *Ae. atropalpus* is more commonly found in warm and unshaded containers (Andreadis and Wolfe 2010; Byrd et al. 2019). The early colonization of rock pools by *Ae. j. japonicus* may result in priority effects, where the abundances of taxa that colonize rock pools after *Ae. j. japonicus* are negatively impacted by the presence of an allopatrically evolved species (Zee and Fukami 2018).

To create an experimental environment that properly reflects natural conditions is often quite challenging, and is likely impossible when natural conditions have not yet been described. To develop a better understanding of the competitive interactions in rock pools, our knowledge of the ecology of common rock pool taxa must be improved. The following study was conducted in two parts: (1) a descriptive 12-month observational field study of southeastern U.S. rock pool community composition and (2) a laboratory experiment analyzing the influence of temperature on the fitness of *Aedes atropalpus*, with the unified goal of addressing important gaps in the fundamental knowledge of rock pool mosquito ecology. The observational study (Aim 1) resulted in the identification of ecologically important water temperatures that correlated with

mosquito species compositions, and those observations were used to inform temperature treatments in the laboratory experiment with *Ae. atropalpus* (Aim 2).

AIM 1: DETERMINE THE ANNUAL PHENOLOGY OF ROCK POOL MOSQUITOES

Rock pools on the Chattooga River in the southeastern U.S. are intriguing for studying the distributions of *Ae. atropalpus* and *Ae. j. japonicus*. Near the river's headwaters in Cashiers, NC, pool temperatures are low, and *Ae. atropalpus* is found in low abundances as compared to a downstream lower elevation site near Clayton, Georgia, where *Ae. j. japonicus* is rarely encountered in the warm water rock pools in the summer (Byrd et al. 2019). Temperature is a strong predictor of the presence or absence of *Ae. j. japonicus* and *Ae. atropalpus* in southeastern rock pools (Byrd et al. 2019), but the seasonality of these species and the commonality of competitors and predators remain largely undescribed in the region, which limits the potential for informed hypotheses to be tested in the field. To strengthen our foundational understanding of rock pool mosquito ecology, Aim 1 of this study was to describe the annual phenology of rock pool macroinvertebrates, focusing primarily on mosquito abundance and species composition. We used observations made in Aim 1 in conjunction with the results of Byrd et al. (2019) to determine the temperature ranges used in Aim 2.

Methods

Study Site

To conduct the observational study we selected rock pools at two sites studied by Byrd et al. (2019) on the Chattooga river: the Bull Pen (BP) rapid in Jackson County, North Carolina (35.015934, -83.126434) at an elevation of 728 meters, and the Bull Sluice (BS) rapid in Rabun County, Georgia (34.817710, -83.303943) at an elevation of 361 meters (Figure 1). We haphazardly selected eight rock pools for sampling at BP and BS, but only sampled six at BS following the first sampling event (Table 1). Our intention was to choose pools comprising a variety of sizes and distances from the river that were safely accessible. The sampling period ran from November 2018 to November 2019 with the initial objective of sampling monthly at both sites. Safety concerns due to adverse weather conditions prevented us from sampling in some months, resulting in only ten sampling events at BP and nine events at BS.

Sampling Method

To consistently sample rock pools throughout the study period we developed a standardized protocol. Samplers used a tape measure to measure the length, width, depth, and distance between the water surface and the lip of each rock pool to the nearest tenth of an inch (converted to centimeters), and used an Oakton pH tester model WD-35634-30, (Vernon Hills, IL) to measure the temperature, conductivity, and pH of the water in the pool. To sample the macroinvertebrate fauna of each pool, samplers performed ten sweeps with a fine mesh aquarium net (3.5 x 2.5 x 0.5 inches) with a single sweep being defined as moving the net swiftly through the water approximately 25 centimeters. The first three sweeps were near the water surface with

Figure 1. Map of the northern Chattooga river highlighting the headwaters and the two rock pool sampling sites. Bull Pen is higher in elevation (728m) with colder rock pool temperatures than Bull Sluice (361m).

the net fully submerged. The next three sweeps were conducted at a middle depth, the following three at the deepest depth of the pool without collecting substrate, and one final sweep was performed to collect substrate at the bottom of the pool. Some pools were too shallow to identify unique sampling depths or to fully submerge the net in water (e.g. Bull Sluice pool 2), and in those cases the protocol was modified to sweep more of the pool's surface area without sampling at different depths. After each sweep, macroinvertebrates were counted and identified to the lowest feasible taxon. Except for mosquitoes, captured invertebrates were held in a separate tray and placed back in the pools after the final sweep. For sampling events prior to May 2019, we identified 2^{nd}-4^{th} instar mosquito larvae

Table 1A. Bull Pen pool measurements taken on 12 January 2019.

Pool Number	Length (cm)	Width (cm)	Depth (cm)	Height to River (cm)
1	63.5	44.5	71	61
2	81	79	85	76
3	150.5	69	69	76
4	55	27	34	30.5
5	63.5	40.6	40.6	91
6	61	35.5	66	91
7	47	28	15	84
8	71	46	23	30.5

Table 1B. Bull Sluice pool measurements taken on 15 January 2019.

Pool Number	Length (cm)	Width (cm)	Depth (cm)	Height to River (cm)
1	85	76	28	366
2	163	90	9	396
3	84	27	26	183
4	56	52	34	122
5	155	71	23	91
6	99	56	20	122
*7	92	70	18	NA
*8	110	94	23	NA

*Only sampled and measured in November 2018.

in the field and returned 1st instar larvae and pupae to the laboratory for identification. After May 2019, all immature mosquitoes were returned to the laboratory alive for identification. In any case, all mosquitoes were identified to species microscopically with morphological characters from a key to larvae of the mid-Atlantic region (Harrison et al. 2016).

First instar larvae of *Ae. j. japonicus* and *Ae. atropalpus* cannot be reliably distinguished with morphological characters, so we reared them to later developmental stages at 27 ºC with a 16:8 light: dark photoperiod in a plant growth chamber (Thermo Scientific model pr505755L; Waltham, MA). Upon emergence we held adults with cotton balls soaked in a 10% sucrose solution and froze them after 72 hours. We removed one wing from each adult female and measured them with a digital microscope camera (Motic Images Plus 3.0; Richmond, British Columbia). We held first instar mosquitoes were for 72 hours before identifying them at later instars.

Statistical Methods

We performed all statistical analyses with R version 3.6.1 (R Core Team 2019) in the RStudio integrated development environment version 1.2.5019 (RStudio Team 2019). We used the R package ggplot2 version 3.2.1 (Wickham 2016) to generate figures and the package lme4 version 1.1-21 (Bates et al. 2015) to compare average pH, conductivity, and mosquito wing length among pools with generalized linear mixed-effect models. To perform Tukey's contrasts for those models we used emmeans version 1.4.2 (Lenth 2019). We used the package cooccur version 1.3 (Griffith et al. 2016) to analyze cooccurrence of mosquito species with a probabilistic model allowing for pairwise comparisons of species and to calculate standardized effect sizes (expected cooccurrence minus observed cooccurrence frequency) (Veech 2013). An effect size <

0 implies a negative association, an effect size of 0 implies no association, and an effect size > 0 implies a positive association between two species.

Results

Abiotic Data

The selected rock pools at both sites varied in depth, surface area, and morphology (Table 1, Figure 2). Based on visual observations at varying river levels, pools with a tendency to become submerged by the river after heavy rainfall had little or no substrate while other pools contained a relatively deep layer of sand, soil, and organic matter. Conductivity and pH varied widely among pools on individual sampling events at BP and BS (Table 2, Table 3). Average conductivity was generally higher at BP than at BS, but individual pools at BS had sometimes accounted for the highest conductivities of the study. There was no significant difference in conductivity or pH among the pools at BP or BS across all sampling events (Table 4). The range of water temperatures throughout the year was greater at BS (4.11 ^{o}C - 34.44 ^{o}C) than BP (7.55 ^{o}C - 22.24 ^{o}C). May 2019 was the first time that late instar *Ae. atropalpus* larvae were encountered at BS (no sampling was done at the site in April), and the average temperature of the rock pools on that date was 23.75 ^{o}C, higher than the average pool temperature on any sampling event at BP.

Wing Lengths of Mosquitoes Collected from Rock Pools

Female *Ae. j. japonicus* emerged from pupae collected from Bull Pen rock pools in the months of May, July, August, and September, with wing lengths ranging from 3.06 – 4.37 mm (Table 5, Figure 3). Mean wing length was the lowest in July and the highest in September. The mean wing length of *Ae. j. japonicus* did not differ among pools across the entire sampling period ($F_{(1,7.03)} = 1.99$, Cohen's $f = 0.15$, $p = 0.20$) but did differ among sampling dates ($F_{(1,97.97)} = 24.38$, Cohen's $f = 0.51$, $p < 0.01$) and diverged among pools over the course of the

study as evidenced by a significant interaction between wing length by pool and sampling date ($F(1,97.79) = 8.92$, Cohen's $f = 0.15$, $p < 0.01$). Although mean *Ae. j. japonicus* wing length did not differ among pools across the study period, analysis of wing lengths from May when the most *Ae. j. japonicus* pupae were collected showed that wing lengths differed significantly among pools on that single sampling event ($F(1,67) = 48.52$, $p < 0.001$).

Female *Ae. atropalpus* emerged from pupae collected from Bull Sluice rock pools in the months of May, June, July, and August had wing lengths ranging from 2.20 mm to 3.28 mm (Table 6, Figure 4). Mean wing length was the smallest in May and the largest in July. There was a significant difference in wing length among pools across the study period ($F(1,56.95) = 5.22$, Cohen's $f = 0.27$, $p = 0.03$) but not among sampling dates ($F(1,77.00) = 24.38$, Cohen's $f = 0.20$, $p = 0.10$). Wing length diverged among pools over the course of the study period ($F(1,76.97) = 8.92$, Cohen's $f = 0.27$, $p < 0.05$).

Species Composition and Abundance

Five mosquito species were collected over the course of the sampling period: *Ae. atropalpus*, *Ae. j. japonicus*, *Culex territans* (Walker), *Cx. restuans* (Theobald), and *Anopheles punctipennis* (Say). Mosquito larvae of at least one species were collected on every sampling event. The relative abundances of mosquito species differed between months and between the two sites (Figure 5). At BP, eight pools were resampled across ten sampling events for a total of 80 individual observations. *Aedes j. japonicus* was present at BP on every sampling event and encountered in 82.5% of the individual observations. *Culex territans* appeared at Bull Pen in April 2019 and was collected every month until November 2019 and was also collected in November 2018, appearing in 48.8% of the total observations. *Culex restuans* was encountered at BP in May 2019 – September 2019, and in November 2019, and was collected on 27.5% of

the total observations. *Anopheles punctipennis* was only collected at BP in May and September at low abundances (Table 7) and was encountered in 7.5% of the individual observations. Four *Ae. atropalpus* larvae were collected at BP in the same pool in June 2019, the only time the species was encountered at the site. The presence of *Cx. territans* was positively associated with the presence of *Cx. restuans* (effect size = 0.11, $p < 0.001$).

At BS, six pools were resampled on nine sampling events (with two additional pools sampled on the first event) for a total of 56 observations. *Aedes atropalpus* was collected on every sampling event except for January 2019 and was encountered in 62.5% of the individual pool observations. *Aedes j. japonicus* was encountered at BS on all nine sampling events and 53.6% of the individual pool observations. *Culex territans* was collected at BS in every month except for January and November 2019 and was encountered in 69.6% of the individual observations, more than any other species at the site. *Culex restuans* and *An. punctipennis* were collected less often and in lower abundances (Table 8), with *Cx. restuans* being encountered in 10.1% of the observations and *An. punctipennis* in 5.4%. Bull Sluice pools contained more mosquitoes on average from May to August than the BP pools, but BP pools contained more in November 2018, January 2019, and November 2019. *Aedes atropalpus* first appeared in the first instar life stage at BS in March alongside larger abundances of *Ae. j. japonicus* and *Cx. territans*. The relative abundances of the two species shifted in subsequent sampling events so that *Ae. atropalpus* was the most abundant at the site by May (Figure 6).

Total mosquito abundance peaked at BP in May with 1,198 mosquitoes, but fell to 175 in the following month, steadily increasing again in subsequent collections to 766 in September, before decreasing in November. Pupae were collected in May, June, July, August, and September. Late instar larvae were encountered on every sampling date except for February and

November 2019. At BS, the peak in mosquito abundance occurred in March 2019 with 1,512 mosquitoes collected from the six rock pools. The abundance of mosquitoes declined in each subsequent collection. Late instar larvae and pupae were encountered at BS on every sampling date except 12 January 2019, but only outnumbered early instar larvae in May and July.

Mosquitoes were the most abundant macroinvertebrate encountered during the study. In all collections combined, 10,494 mosquitoes were collected. The second-most abundant were Chironomidae, with 979 encountered in total (Table 9). Although mosquitoes were the most abundant family overall, Chironomidae and nematodes were more abundant in January 2019 and November 2019 respectively at the BS site (Figure 7). Across the entire study at both sites we identified twelve orders of macroinvertebrates and encountered at least 18 families. Among those taxa were several predators of mosquitoes and other invertebrates, including water striders (Gerridae), broad-shouldered water striders (Veliidae), backswimmers (Notonectidae), dragonflies (Odonata), riffle beetles (Elmidae), diving beetles (Dytiscidae), and phantom midges (Chaoboridae). We found no evidence of cooccurrence (positive or negative) between mosquitoes and any predacious taxa in our data.

Discussion

Abiotic Data

While no consistent differences between pools was seen at either site, the large ranges of conductivity and pH between pools on individual sampling dates demonstrate that closely located rock pools can have markedly different water chemistry parameters. At both sites, single sampling events saw observations of pools with very a wide range of conductivities and pH levels. It is unclear whether such differences may impact community composition in rock pools, but there is evidence that water chemistry affects mosquito ovipositional tendencies (Bently and Day 1989; Li et al. 2009; Allgood and Yee 2017).

Mosquito Wing Lengths

Our results indicate that wing lengths of *Ae. atropalpus* and *Ae. j. japonicus* can vary significantly among pools in pupae collected on a single sampling date, but that the difference among pools is not consistent over time, as pupae collected from the same pools had different mean wing lengths on different sampling dates. This likely means that habitat conditions within a rock pool vary temporally. An individual rock pools may be preferable for larval development in comparison at one point in time, but detrimental to population growth at another time. The wing lengths reported here can serve as a record of body sizes for these species when emerging naturally from rock pools, which will be useful when determining whether experimental conditions are too harsh. For example, Armistead et al. (2008) measured wing lengths of *Ae. atropalpus* smaller than 2.0 mm on average and wing lengths of *Ae. j. japonicus* less than 2.5 mm on average in all treatments. Those body sizes were so small that the finite population growth rate of *Ae. atropalpus* was calculated as zero in all treatments, causing the researchers to

postulate that their experimental conditions were unrealistically harsh. The wing lengths of the mosquitoes in that experiment were much smaller than those seen in this observational study, indicating that conditions were in fact harsher than natural conditions.

Mosquito Species Compositions at Bull Pen and Bull Sluice

Like *Ae. atropalpus*, *Ae. j. japonicus* females lay diapausing eggs that overwinter in the embryonic phase as a response to shortened days and low temperatures (Bova et al. 2019). Overwintering larvae have also been observed (LaCasse and Yamaguti 1950; Armistead et al. 2012), specifically as 3rd and 4th instars in New Jersey (Scott 2003). The early appearance of the species in aquatic habitats is recognized as a potential competitive advantage, but it is unclear whether the reported observations of early season *Ae. j. japonicus* larvae were true detections of early season hatching or a consequence of overwintering larvae (Kaufman and Fonseca 2014). The data in this study suggest that hatching of *Ae. j. japonicus* occurred under winter conditions in January or February, as indicated by the sharp increase in *Ae. j. japonicus* abundances in BP rock pools during that time. A low number of first instar *Ae. j. japonicus* larvae were also detected in three of the six BS pools in January followed by high abundances of the species in March. These notable increases in *Ae. j. japonicus* abundance during winter months provide evidence that early season hatching is the primary method for obtaining an "early start" instead of overwintering as larvae.

Bull Sluice rock pools had high average abundances of *Ae. j. japonicus* and *Cx. territans* in March 2019 when 1st instar *Ae. atropalpus* larvae were detected for the first time that year, creating the potential for stage-dependent competition between late instar *Ae. j. japonicus* and early instar *Ae. atropalpus* in rock pools. There is some evidence that competition between early and late instar mosquito larvae can have exaggerated impacts on the younger individuals as

compared to the impacts of same-stage competition, probably because of resource competition but possibly because of intraguild predation (Edgerly et al. 1999; Lounibos et al. 2003). This observation presents an opportunity to study the impact of stage-dependent competition with realistic species compositions. Competition experiments with mosquitoes, including those with *Ae. j. japonicus* and *Ae. atropalpus*, traditionally use synchronous hatching so that the mosquitoes in the experiment are the same age (Armistead, Nishimura, et al. 2008b; Armistead, Arias, et al. 2008; Alto 2011; Hardstone et al. 2012; Freed et al. 2014). In the case of *Ae. j. japonicus*, the true extent of its competitive interactions may be better understood through exploration of its competitive interactions as late-instar larvae in the presence of new hatchlings, as is observed in the early season (Kaufman and Fonseca 2014).

Fewer BP rock pools were sampled in this study than by Byrd et al. (2019), and it is possible that the haphazard pool selection resulted in sampling of pools at BP that are less likely to attract oviposition by *Ae. atropalpus*. Still, in the pools sampled at BP, there were nearly no observed opportunities for interspecific competition between *Ae. j. japonicus* and *Ae. atropalpus*, and Byrd el al. (2019) also observed low abundances of the species at the same site. This may be an important observation, as it leads to two clear hypotheses: (1) that *Ae. atropalpus* has nearly been displaced from the BP site and the surrounding area, or (2) females avoid laying eggs the sampled rock pools. For *Ae. atropalpus* to be considered displaced in BP pools by *Ae. j. japonicus*, there should be evidence that the species would utilize rock pools at the site in the absence of the invasive species. It may be possible that *Ae. atropalpus* has historically avoided these pools, leaving available habitat for *Ae. j. japonicus*. Widespread sampling of mosquito habitats near BP for *Ae. atropalpus* during the summer months would be helpful for determining whether the species is abundant in the area. Studies of ovipositional site selection with *Ae.*

atropalpus would be important for understanding why so few *Ae. atropalpus* are found in these pools.

Overall Abundances of Invertebrate Taxa

Mosquitoes were the most abundant macroinvertebrate taxon with over 10,000 larvae and pupae collected across the entire study. Of the taxa collected, Odonata, Veliidae, Gerridae, Notonectidae, Elmidae, and Dytiscidae are the potential predators of mosquitoes (Mirua and Takahashi 1988; Ohba et al. 2011; Saha et al. 2012; Roux et al. 2015). Mosquitoes seem likely to play an important role in the ecology of southeastern rock pools. In this study they were present as larvae year-round, inhabiting rock pools with high relative abundances early in the season (Figure 7). For predaceous macroinvertebrates like dragonfly nymphs, mosquito larvae and pupae may serve as crucial food sources.

Conclusion

Mosquitoes were more abundant than any other macroinvertebrate taxa in the sampled pools. A thorough understanding of their ecology is important to understanding the broader ecology of the rock pool system. *Aedes j. japonicus* was the most abundant mosquito in the cool-water rock pools near the high-elevation rock pools at the Bull Pen (BP) on the Chattooga River where the native rock pool mosquito, *Ae. atropalpus,* is rarely encountered. The low detection rate of *Ae. atropalpus* at BP could mean that the species has either already been displaced from the area by invasive *Ae. j. japonicus*, or that *Ae. atropalpus* preferentially avoids oviposition in the sampled pools. To better understand the absence of *Ae. atropalpus* in BP pools, confirmation of the species' sustained present in the surrounding area are necessary. Studies on ovipositional preferences of the native species would also be informative. At a warmer low-elevation site near the Bull Sluice (BS) rapid of the Chattooga River, *Ae. atropalpus* is more common and abundant, especially in the summer months, but *Ae. j. japonicus* is present with high relative abundances early and late in the season. This study also provided evidence that *Ae. j. japonicus* eggs hatch late in the winter as demonstrated by a large increase in larval abundance from January to February at BP. High abundances of early and late-stage *Ae. j. japonicus* in rock pools during the early season may present the greatest competitive challenge to native species, creating a situation where early season *Ae. atropalpus* hatchlings emerge in pools already inhabited by late instar *Ae. j. japonicus*. A logical next step would be to study the impacts of asymmetrical larval competitions at cooler temperatures to estimate the impact of this observation on *Ae. atropalpus* populations.

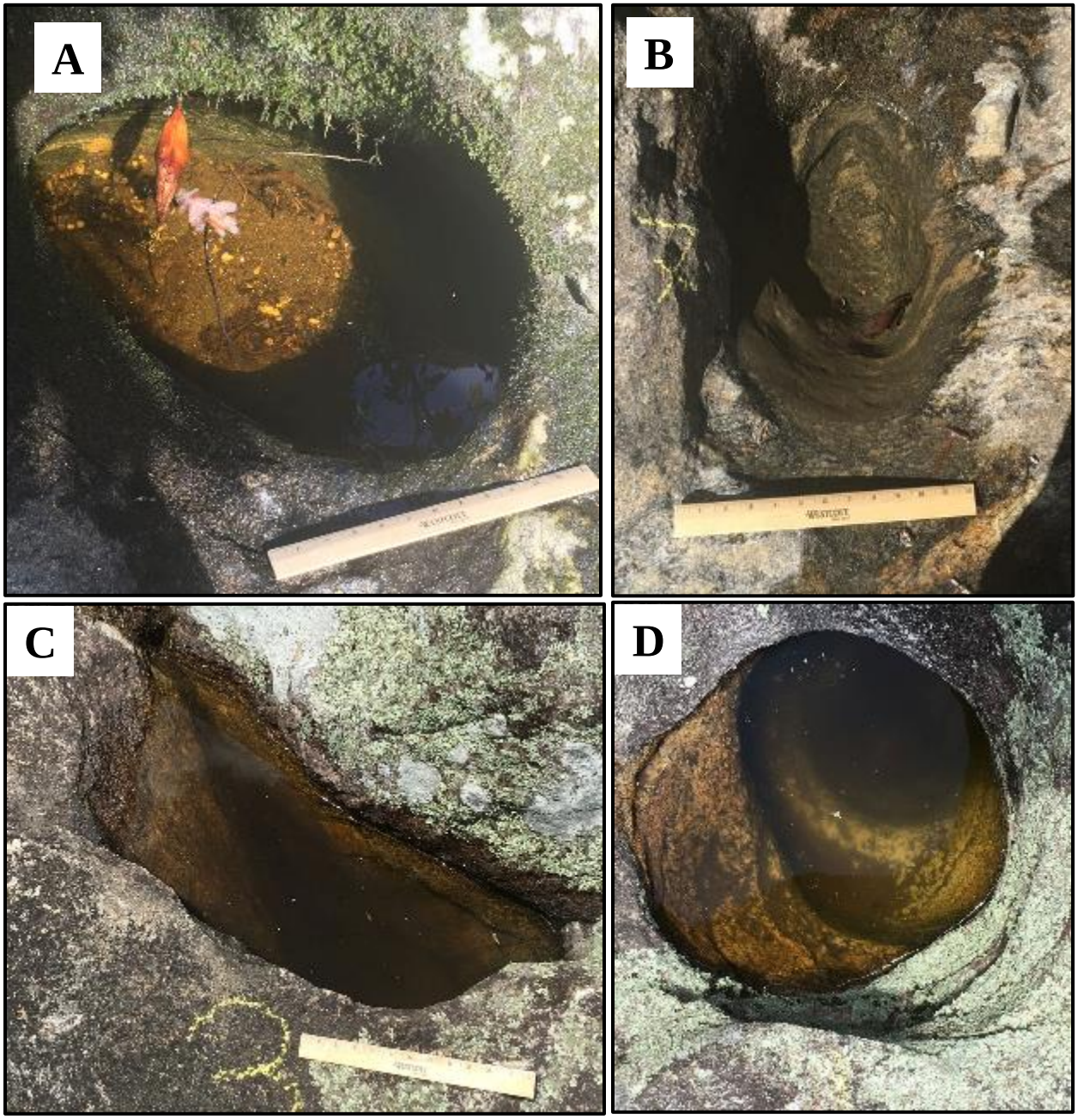

Figure 2. Rock pools 5 and 7 at Bull Pen (A, B) and pools 3 and 4 at Bull Sluice (C, D).

Table 2. Summary statistics for monthly water chemistry data at Bull Pen. Mean values and range are reported for each sampling date.

Date	Temperature (°C)	Conductivity (µS)	pH
11/5/2019	13.24 (12.0 – 14.0)	27.32 (23.1 – 30.1)	7.25 (6.57 – 7.65)
1/15/2019	7.55 (4.8 – 10.6)	20.74 (11.6 – 32.6)	7.97 (7.95 – 9.11)
2/17/2019	7.79 (7.1 – 8.2)	17.93 (11.3 – 21.5)	8.26 (8.01 – 8.79)
4/27/2019	14.18 (12.1 – 18.6)	23.1 (13.4 – 42.8)	8.35 (7.85 – 9.63)
5/20/2019	18.30 (16.9 – 20.0)	25.93 (15.4 – 34.9)	7.63 (6.76 – 8.05)
6/22/2019	19.97 (18.9 – 20.9)	Not Measured	Not Measured
7/31/2019	19.7 (18.7 – 21.7)	22.7 (15.9 – 29.6)	7.42 (6.76 – 8.21)
8/31/2019	17.75 (17.0 – 18.7)	36.99 (26.2 – 65.5)	6.94 (6.35 – 7.59)
9/28/2019	22.24 (20.3 – 23.5)	70.4 (48.3 – 123.9)	6.85 (6.50 – 7.39)
11/8/2019	13.24 (12.0 – 14.0	27.32 (23.1 – 30.1)	7.25 (6.57 – 7.65)

Table 3. Summary statistics for monthly water chemistry data at Bull Sluice. Mean values and range are reported for each sampling date.

Date	Temperature (°C)	Conductivity (µS)	pH
11/3/2018	9.38 (7.9 – 10.8)	7.69 (4.3 – 11.9)	7.16 (6.02 – 9.01)
1/12/2019	4.11 (3.0 – 5.7)	6.07 (2.3 – 12.7)	7.19 (4.00 – 8.56)
3/23/2019	15.6 (10.4 – 20.3)	10.63 (7.3 – 13.5)	7.06 (6.38 – 8.63)
5/10/2019	23.75 (23.2 – 24.7)	14.65 (7.7 – 27.7)	6.76 (6.18 – 7.06)
6/12/2019	25.3 (23.7 – 26.0)	8.10 (4.6 – 16.2)	7.13 (6.73 – 7.84)
7/11/2019	34.44 (31.6 – 37.6)	14.03 (5.4 – 45.4)	7.29 (6.39 – 9.88)
8/24/2019	29.42 (28.3 – 30.4)	54.52 (10.9 – 156.7)	7.78 (6.39 – 9.88)
9/14/2019	27.42 (26.7 – 28.0)	65.52 (15.2 – 174.1)	7.18 (5.85 – 9.92)
11/3/2019	12.13 (9.5 – 17.3)	8.73 (3.3 – 17.3)	7.66 (7.15 – 8.53)

Table 4. Results of generalized linear mixed-effect models comparing mean conductivity and pH among pools at BP and BS across the entire study period with pool as a random effect.

Site	Variable	F	d.f.	p-value
Bull Pen	Conductivity	1.31	1, 66	0.26
	pH	0.93	1, 6.19	0.37
Bull Sluice	Conductivity	0.18	1, 8.24	0.68
	pH	0.009	1, 8.43	0.93

Table 5. Average wing lengths of female *Aedes japonicus japonicus* collected as pupae at Bull Pen.

Month	n	Mean Wing Length (mm) (Min – Max)
May	69	3.84 (3.30 – 4.24)
July	5	3.40 (3.06 – 3.61)
August	23	3.58 (3.24 – 4.13)
September	5	4.10 (3.88 – 4.37)

Table 6. Average wing lengths of female *Aedes atropalpus* collected as pupae at Bull Sluice.

Month	n	Mean Wing Length (mm) (Min – Max)
May	6	2.51 (2.2 – 3.08)
June	25	2.78 (2.47 – 2.97)
July	28	2.84 (2.21 – 3.28)
August	22	2.76 (2.42 – 3.08)

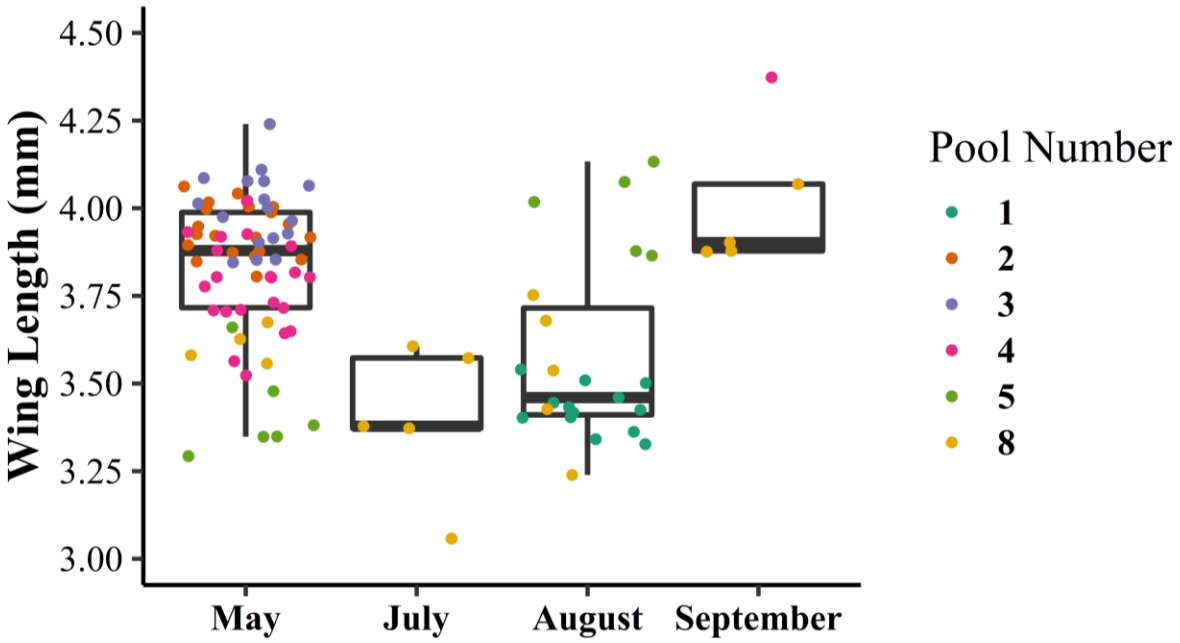

Figure 3. *Aedes j. japonicus* wing length by month measured from pupae collected at Bull Pen.

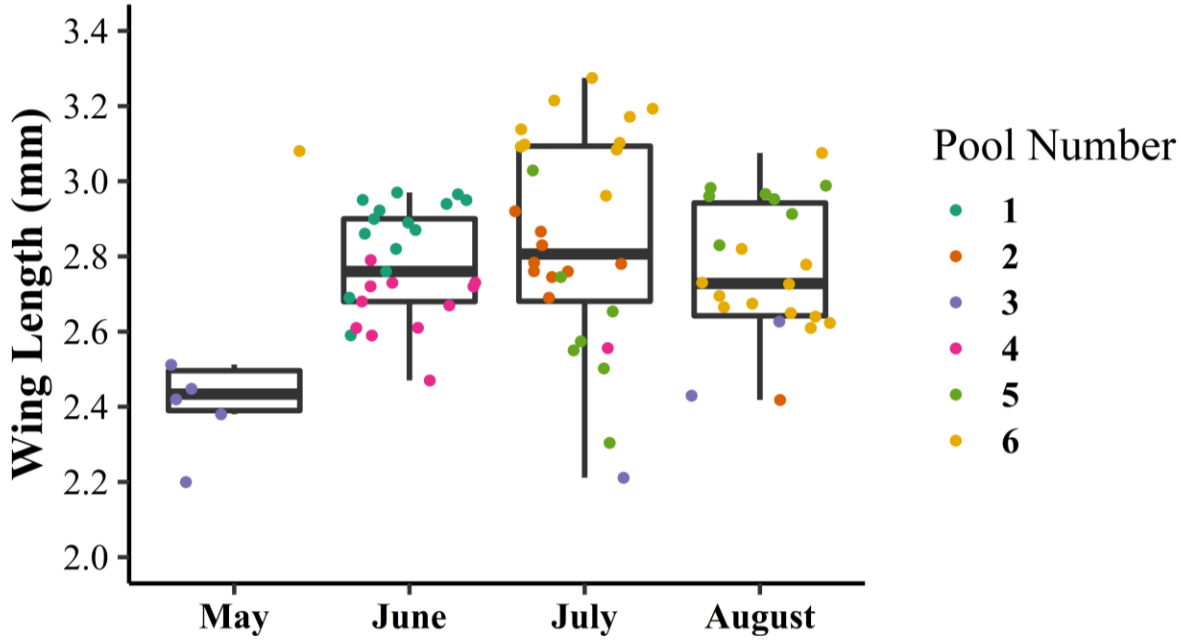

Figure 4. *Aedes atropalpus* wing length by month measured from pupae collected at Bull Sluice.

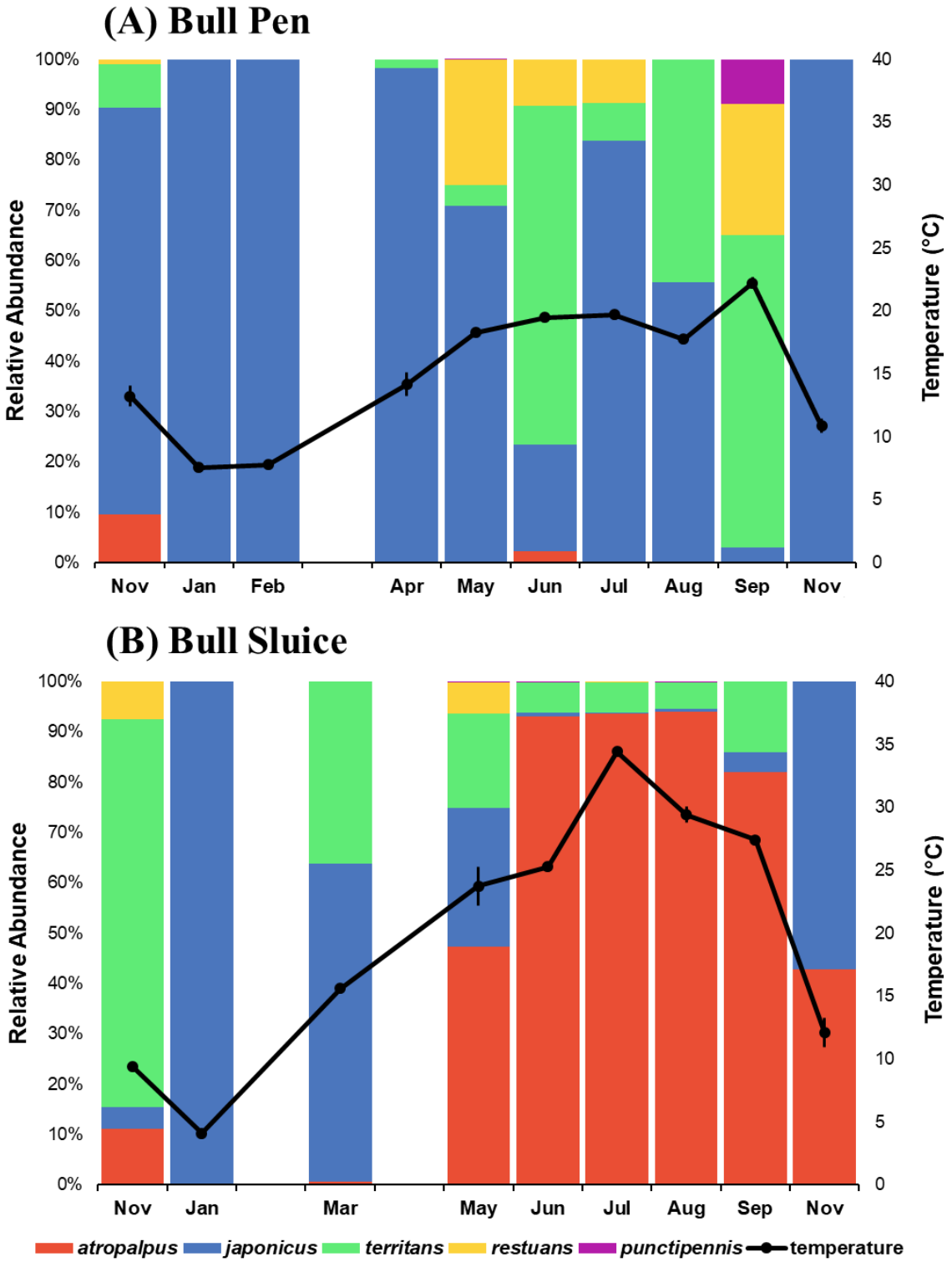

Figure 5. Monthly mosquito species compositions and average rock pool temperatures at time of sampling at (A) Bull Pen and (B) Bull Sluice.

Table 7. Mean abundances of mosquitoes per pool at Bull Pen (BP).

Date	Mean Total (SE)	*Aedes atropalpus* (SE) (proportion of pools observed)	*Aedes j. japonicus* (SE) (proportion of pools observed)	*Culex territans* (SE) (proportion of pools observed)	*Culex restuans* (SE) (proportion of pools observed)	*Anopheles punctipennis* (SE) (proportion of pools observed)
11/5/2018	18.80 (± 5.83)	0	16.80 (± 5.33) (4 / 5)	1.80 (± 0.73) (4 / 5)	0.20 (± 0.20) (1 / 5)	0
1/15/2019	8.0 (± 2.68)	0	8.0 (± 2.68) (7 / 8)	0	0	0
2/17/2019	60.25 (± 10.87)	0	60.25 (± 10.87) (8 / 8)	0	0	0
4/27/2019	21.75 (± 5.34)	0	21.25 (± 5.30) (8 / 8)	0.38 (± 0.18) (3 / 8)	0	0
5/20/2019	135.50 (± 23.31)	0	93.38 (± 17.73) (8 / 8)	5.37 (± 2.21) (6 / 6)	32.75 (± 12.32) (7 / 8)	0.13 (± 0.13) (1 / 8)
6/22/2019	21.88 (± 15.28)	0.50 (± 0.50) (1 / 8)	3.375 (± 2.27) (5 / 8)	14.75 (± 9.87) (6 / 8)	2.00 (± 2.00) (1 / 8)	0
7/31/2019	43.87 (± 19.93)	0	36.13 (± 20.26) (8 / 8)	3.25 (± 1.42) (5 / 8)	3.75 (± 3.20) (3 / 8)	0
8/31/2019	74.75 (± 33.61)	0	40.50 (± 32.01) (6 / 8)	32.13 (± 14.16) (7 / 8)	0	0
9/28/2019	133.13 (± 77.63)	0	4.00 (± 2.06) (3 / 8)	81.625 (± 37.11) (8 / 8)	34.38 (± 39.50) (6 / 8)	11.625 (± 9.07) (5 / 8)
11/8/2019	11.00 (± 3.21)	0	11.00 (± 3.21) (7 / 8)	0	0	0

Table 8. Mean abundances of mosquitoes per pool at Bull Sluice (BS).

Date	Mean Total (SE)	*Aedes atropalpus* (SE) (proportion of pools observed)	*Aedes j. japonicus* (SE) (proportion of pools observed)	*Culex territans* (SE) (proportion of pools observed)	*Culex restuans* (SE) (proportion of pools observed)	*Anopheles punctipennis* (SE) (proportion of pools observed)
11/3/2018	11.75 (± 6.52)	1.25 (± 0.77) (3 / 7)	0.50 (± 0.33) (2 / 7)	9.13 (± 6.6) (6 / 7)	0.88 (± 0.88) (1 / 7)	0
1/12/2019	2.0 (± 0.93)	0	2.0 (0.93) (3 / 6)	0	0	0
3/23/2019	252.0 (± 82.63)	0.50 (± 0.22) (3 / 6)	38.33 (± 16.70) (5 / 6)	21.83 (± 6.62) (6 / 6)	0	0
5/10/2019	215.00 (± 55.94)	75.00 (± 24.19) (5 / 6)	43.83 (± 31.68) (6 / 6)	29.67 (± 8.85) (6 / 6)	9.67 (± 6.07) (3 / 6)	0.33 (± 0.33) (1 / 6)
6/12/2019	187.83 (± 65.20)	119.33 (± 43.81) (5 / 6)	0.83 (± 0.31) (4 / 6)	7.67 (± 1.78) (5 / 6)	0.17 (± 0.17) (1 / 6)	0.17 (± 0.17) (1 / 6)
7/11/2019	111.29 (± 58.10)	101.71 (± 58.98) (6 / 6)	0.29 (± 0.18) (2 / 6)	6.57 (± 4.05) (4 / 6)	0.14 (± 0.14) (1 / 6)	0
8/24/2019	177.50 (± 65.08)	159.67 (± 58.03) (6 / 6)	0.83 (± 0.65) (2 / 6)	9.17 (± 2.57) (6 / 6)	0	0.17 (± 0.17) (1 / 6)
9/14/2019	80.50 (± 35.47)	45.83 (± 32.64) (6 / 6)	2.17 (± 1.38) (2 / 6)	7.83 (± 3.77) (5 / 6)	0	0
11/3/2019	4.50 (± 2.13)	2.00 (± 1.29) (2 / 6)	2.67 (± 1.14) (3 / 6)	0	0	0

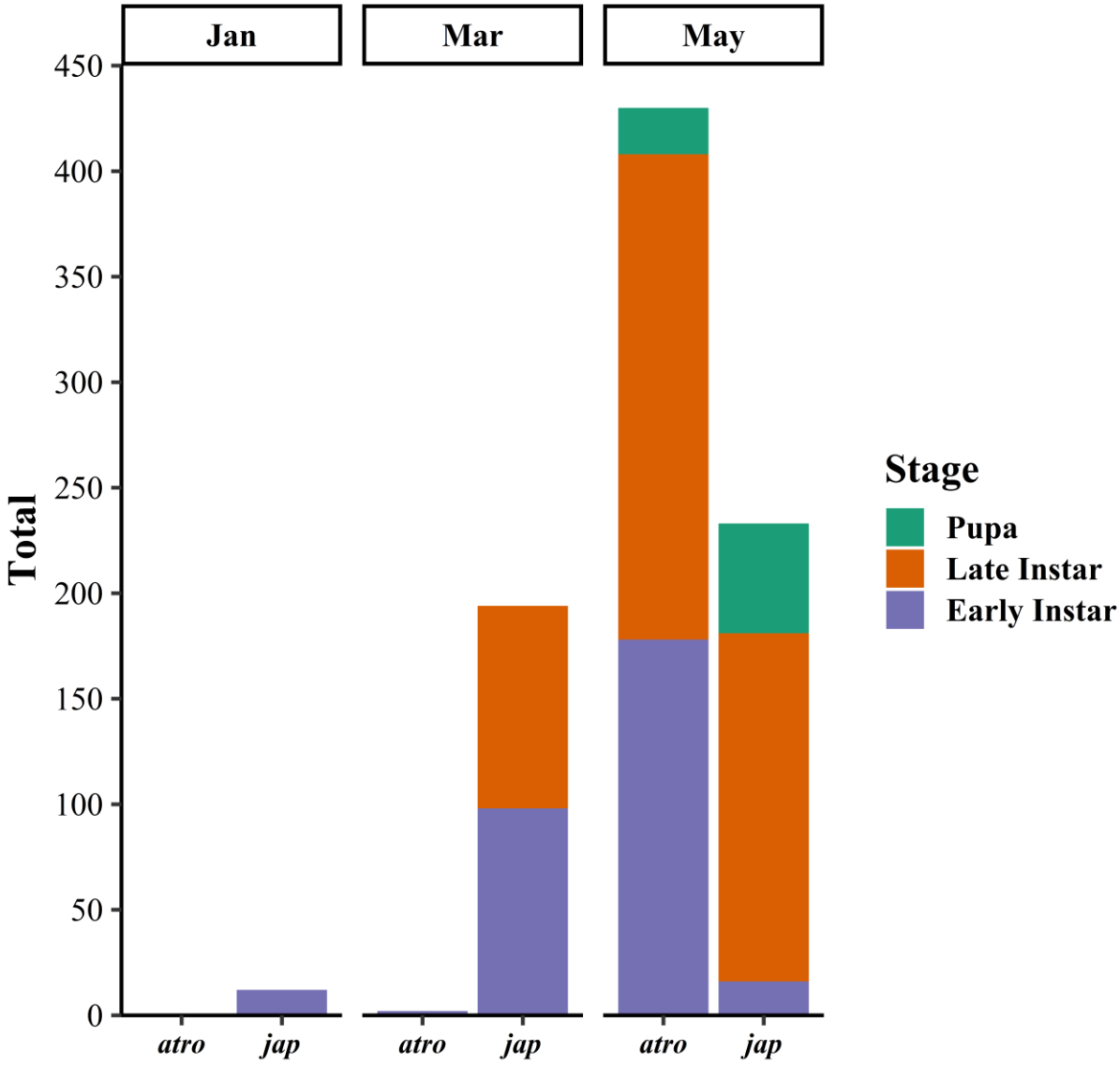

Figure 6. Relative abundances of *Aedes atropalpus* and *Ae. j. japonicus* in the early season at Bull Sluice. Early instar refers to 1st and 2nd instar larvae and late instar refers to 3rd and 4th instar larvae.

Table 9. List of taxa and the numbers of individuals observed during the sampling period.

Order	Family	Number of Observations	Sites Collected
Diptera	Culicidae	10,514	BP + BS
	Chironomidae	979	BP + BS
	Chaoboridae	7	BP
Nematoda	Unknown	214	BS
Hempitera	Veliidae	64	BP + BS
	Gerridae	9	BP
	Notonectidae	5	BS
Odonata	Libellulidae	26	BS
	Unknown	12	BP
Ephemeroptera	Unknown	14	BP + BS
Coleoptera	Elmidae	5	BP + BS
	Dytiscidae	2	BP + BS
	Unknown	1	BP
Annelida	Unknown	6	BP
Trichoptera	Unknown	6	BP + BS
Plecoptera	Taeniopterygidae	4	BP
Decapoda	Cambaridae	2	BP
Anura	Unknown	1	BP
Acari	Unknown	Did not count	BP + BS

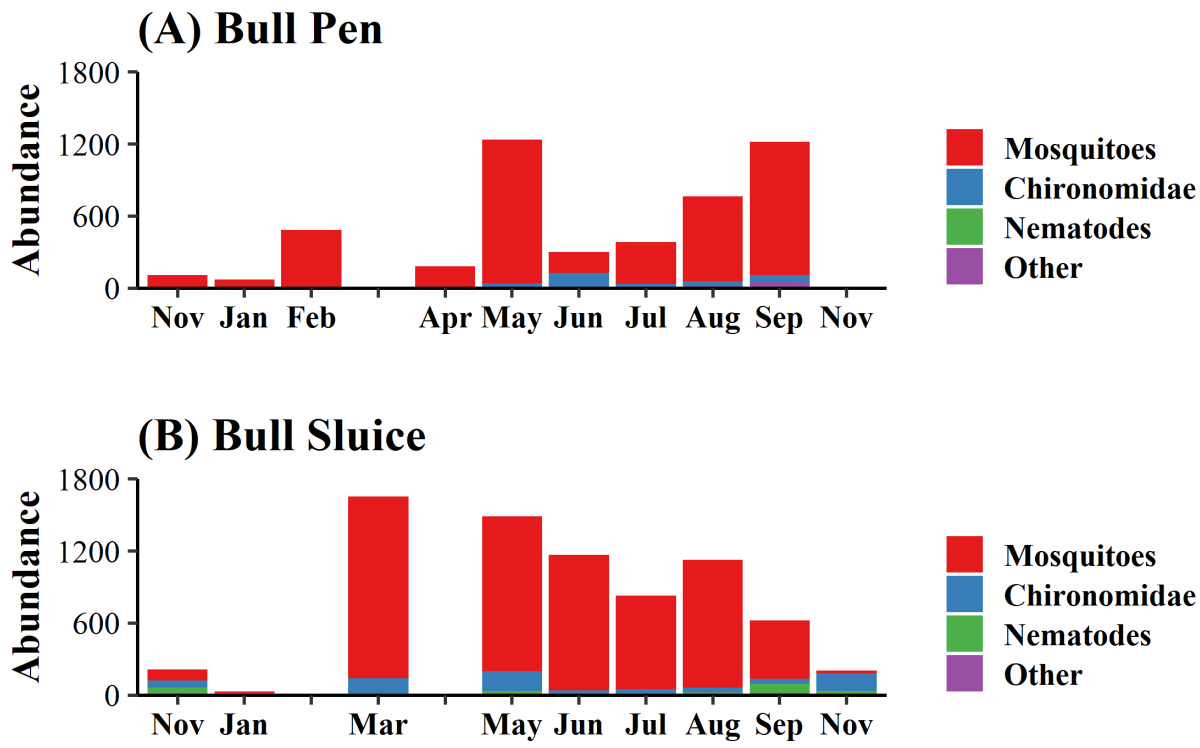

Figure 7. Monthly macroinvertebrate abundance at Bull Pen and Bull Sluice.

AIM 2: ANALYZE THE FITNESS OF AUTOGENOUS *AEDES ATROPALPUS* REARED AT DIFFERENT TEMPERATURES

Aim 2 of this study was to analyze the fitness of *Ae. atropalpus* raised to adulthood at three non-overlapping temperature ranges. The first hypothesis was that developmental temperature would affect the size-fecundity relationship of autogenous *Ae. atropalpus*, with warmer temperatures resulting in stronger linear relationships between wing length and fecundity as evidenced by a larger slope. The size-fecundity relationship is sometimes assumed to be static in mosquitoes, and functions relating size and fecundity are regularly taken from the literature for use in estimating the fitness of mosquitoes in a different study (Juliano 1998; Alto et al. 2008; Armistead, Arias, et al. 2008; Armistead, Nishimura, et al. 2008a; Freed et al. 2014; Costanzo et al. 2018). The only study of environmentally induced variation in the size-fecundity relationship in mosquitoes found that the strength of the linear relationship increases with temperature in *Ae. albopictus* (Costanzo et al. 2018).

Aedes atropalpus is rarely encountered in cold rock pools (Byrd et al. 2019), and it is unclear whether this is evidence of preferential oviposition by *Ae. atropalpus* in warmer water or a competitive barrier where *Ae. j. japonicus* prevents success in cooler pools. Because *Ae. atropalpus* likely has a competitive disadvantage of increased development time to allow for autogenous reproduction (Armistead et al. 2008), we predicted that faster development time due to high water temperature is beneficial for the species, leading to the second hypothesis of Aim 2, that *Ae. atropalpus* would have improved fitness at higher temperatures.

Methods

Experimental Design

We based the temperature ranges in this study on ecologically important temperatures observed in Aim 1 and by Byrd et al. (2019). At Bull Pen, water temperatures were around 20 °C on average in the summer and few *Ae. atropalpus* larvae were collected at that site in Aim 1 and by Byrd et al. (2019). To determine whether *Ae. atropalpus* can successfully develop in the cool water temperatures at Bull Pen, we chose the lowest temperature range in this experiment to be 16 °C - 20 °C. Most observations of similar relative abundances between *Ae. atropalpus* and *Ae. j. japonicus* were at the temperature range of 23 °C - 27 °C, which overlaps the optimal developmental temperature of *Ae. j. japonicus* (Reuss et al. 2018), so we chose the middle temperature range to be 23 °C - 27 °C. This mid-temperature range would be predicted as favorable to *Ae. j. japonicus*, but *Ae. j. japonicus* was uncommonly observed in pools within that range in Aim 1. We chose the highest temperature range be 30 °C - 34 °C because *Ae. atropalpus* was often found in high relative abundances at that range.

Twenty-four hours prior to beginning the experiment, we placed six BioQuip larval trays (catalog no. 1426B; 34.3 x 25.4 cm) with 900mL of water and 0.5g of bovine liver powder along with six Bioquip pupal rearing chambers (catalog no. 1425; 32 ounces) with 400mL of water in three environmental chambers. We programmed each of the environmental chambers for a 16L:8D photoperiod. A Conviron e8 (Winnipeg, Canada) was set to 20 °C in daytime and 16 °C at nighttime, a Percival i41vl (Perry, Iowa) was set to 27 °C in light and 23 °C in darkness, and a Thermoscientific pr505755L (Waltham, MA) was set to 34 °C in light and 30 °C in darkness. A HOBO Pendant wireless temperature data logger (Onset; Bourne, MA) was placed in each tray and used to calculate the average water temperature of each experimental unit. The grand mean

of the average water temperature of each replicate was 19.13 °C for the 16 °C - 20 °C treatment, 24.9 °C for the 23 - 27 °C treatment, and 31.02 °C for the 30 - 34 °C treatment. The grand mean temperature for each treatment was rounded to the nearest whole number (19 °C, 25 °C, 31 °C) and subsequently used to reference the treatments.

During Aim 1 we collected *Ae. atropalpus* larvae and pupae from Bull Sluice pools and used them to establish a laboratory colony, and after approximately two months we used eggs from that colony for this experiment. Twenty-four hours prior to beginning the experiment, we hatched *Ae. atropalpus* eggs in tap water and held the hatchlings in the 25 °C environmental chamber. After 24 hours, we moved the larvae to spot plates in groups of ten and randomly assigned them to temperature treatments, placing 30 *Ae. atropalpus* hatchlings in each tray. The environmental chambers served as treatments and the larval trays served as experimental units, for a total of three temperature treatments (19 °C, 25 °C, 31 °C) and 18 experimental units. The mosquitoes developed in the trays and upon pupation we transferred them into pupal rearing chambers by cohorts. We placed cotton balls soaked in a 10% sucrose solution on the tops of rearing chambers and covered the cotton balls with plastic cups to prevent dehydration. Each day we removed the adults that emerged in the rearing chambers by removing the upper compartments of the chambers and placing them in the 25 °C environmental chamber with continuous access to sucrose for 36 hours (Figure 8). Most adults moved to the upper compartment on their own to access the sugar water, and we moved those that did not via aspiration. Each day we replaced the upper compartments of the pupal rearing chambers if adults had emerged. After 36 hours we killed the adults by freezing. As an estimate of body size, we measured the wings of adults (Packer and Corbet 1989) with a digital microscope camera (Motic; Richmond, British

Figure 8. Upper compartments of rearing chambers with adult *Aedes atropalpus* in 23°C - 27°C environmental chamber.

Columbia). To estimate fecundity we dissected and counted matured ovarian follicles (Clements and Boocock 1984; Telang and Wells 2004) (Figure 9).

Statistical Methods

We performed all statistical analyses in R version 3.6.1 (R Core Team) in RStudio version 1.2.5019 (RStudio Team) and used the package ggplot 2 version 3.2.1 to generate all figures (Wickham 2016). We conducted least-squares linear regression with fecundity (dependent) and wing length (independent) for all females that emerged during the experiment, and separately for each temperature treatment with fecundity dependent on wing length. To compare the slopes of the wing length-fecundity relationships of females emerging from each temperature treatment we conducted an analysis of covariance (ANCOVA) with fecundity as the response variable, temperature as a categorical predictor, and wing length as a covariate predictor (Costanzo et al. 2018). The null hypothesis of the ANCOVA was that the slopes of the size-fecundity lines were equal among temperature treatments. A p-value less than 0.05

in the interaction term (treatment*wing length) was evidence for the rejection of the null hypothesis.

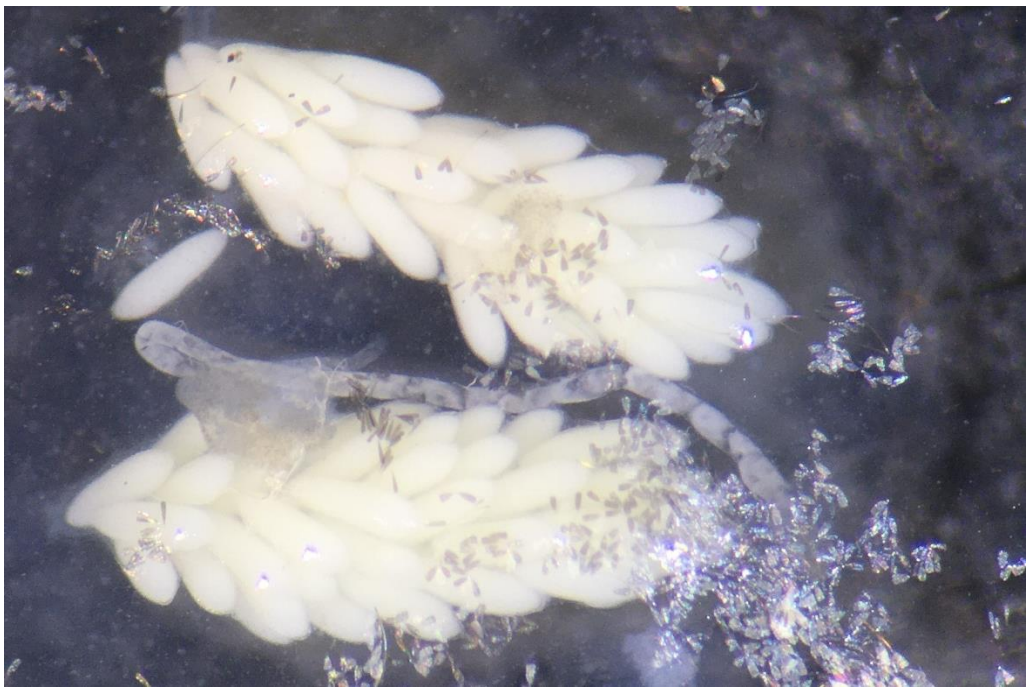

Figure 9. Matured ovarian follicles of autogenous *Aedes atropalpus*.

We conducted a multivariate analysis of variance (MANOVA) to measure differences among four correlated response variables: day of eclosion, wing length, matured ovarian follicles (fecundity), and survival. We conducted a separate MANOVA for males without the fecundity response variable. The null hypothesis of the MANOVA was that the response variables would not differ between rearing temperatures. We calculated mean values for day of eclosion, wing length, and fecundity for each experimental unit and used those in the MANOVA. As a measure of survival, we used the proportion of mosquitoes that emerged as adults from each experimental unit, with the assumption that half of the larvae (n = 15) in each experimental unit were males. In two experimental units more than 15 males emerged (n = 16; n = 17) and in one experimental unit 16 females emerged, resulting in a proportion > 1.0. In those cases, we converted the proportion to 1.0 for the MANOVA analysis.

Because of concerns that the data may violate the assumption of homoscedasticity we used the R package MANOVA.rm version 0.3.4 (Friedrich et al. 2019a; Friedrich et al. 2019b) which performs a MANOVA that does not assume multivariate normality and calculates a modified ANOVA test statistic (MATS) that is appropriate for heteroscedastic data (Friedrich and Pauly 2018). We used the MANOVA.wide() function to calculate the MATS and the simCI() function to calculate pairwise Tukey contrasts for the MANOVA. The simCI() function uses bootstrap values of a summary statistic to calculate multivariate p-values and simultaneous confidence intervals (Friedrich and Pauly 2018).

To compare the impacts of the treatments on overall fitness we used Juliano's (1998) analog of the composite index of population performance (Livdahl and Sugihara 1984) with the following equation:

$$\lambda' = exp\left[\frac{ln[(1/N_0)\sum_x A_x f(w_x)]}{D + [\sum_x xA_x f(w_x)/\sum_x A_x f(w_x)]}\right]$$

where N_0 is the number of females initially within each experimental unit (assumed to be 15), A_x is the number of females eclosing on day x, $f(w_x)$ is a function of fecundity based on wing length for females eclosing on day x, and D is the time from eclosion to reproduction, assumed here to be seven days. We calculated unique functions relating wing length and fecundity for each temperature treatment based on the results of least squares linear regression. We calculated $\boldsymbol{\lambda'}$ for each replicate and compared the grand mean $\boldsymbol{\lambda'}$ for each treatment by conducting an ANOVA.

Results

Comparisons of Size-Fecundity Relationships

There was a significant linear relationship between fecundity and wing length for the females that emerged during the experiment (Table 10). The linear size-fecundity relationships were also significant when considering the females that emerged from the three treatments separately (Table 10, Figure 10). The 19 ^{o}C treatment yielded the lowest y-intercept and the steepest slope, the 25^{o}C treatment yielded the highest y-intercept and the shallowest slope, and the 31 ^{o}C treatment yielded the second-highest y-intercept and the second-steepest slope. There was not sufficient evidence to reject the null hypothesis of no difference between slopes ($F_{(2,161)} = 2.65$, $p = 0.07$). Because it was non-significant, the interaction term was dropped from the ANCOVA model. In the reduced model, both temperature ($F_{(2,161)} = 94.45$, $p < 0.001$) and wing length ($F_{(2,162)} = 127.91$, $p < 0.001$) correlated significantly with fecundity. The significant result in the reduced model was because of differences between the 19 ^{o}C and 31 ^{o}C treatments (Tukey's HSD; $p < 0.001$) and the 19 ^{o}C and 25 ^{o}C treatments (Tukey's HSD; $p < 0.001$), with no significant difference between the 25 ^{o}C and 31 ^{o}C treatments (Tukey's HSD; $p > 0.05$).

Population Growth Correlates

Males emerged the earliest in the 31 ^{o}C treatment followed by the 25 ^{o}C and 19 ^{o}C treatments respectively (Figure 11). Male wing lengths were smallest at the highest temperature, followed by the 25 ^{o}C and 19 ^{o}C temperatures. Survival was highest at 25 ^{o}C, followed by 31 ^{o}C and 19 ^{o}C (Table 11). The response variables differed significantly among the treatments (MATS = 611.138, $p < 0.001$), with pairwise contrasts suggesting that each treatment comparison

contributed to the significant difference (Table 12). Day of eclosion and wing length contributed to the significant result for the male MANOVA, but survival did not (Table 13.

Females also emerged soonest at 31 °C, followed by 25 °C and 19 °C respectively (Figure 11). Wing lengths were smallest at 31 °C, larger at 19 °C, and largest at 25 °C (Table 14). Survival was the greatest at 25 °C, followed by 31 °C and 19 °C respectively. Fecundity was also highest at 25 °C, followed by 31 °C and 19 °C. There was evidence for the rejection the null hypothesis that the multivariate responses did not differ between temperature treatments in females (MATS = 610.011, $p < 0.001$). Pairwise contrasts suggested that a difference between the middle (25 °C) and high (31°C) treatments was the only one contributing to the significant difference among treatments (Table 15). Each response variable contributed to the overall difference among treatments for females (Table 13).

Finite Population Growth Rates

The average finite rate of increase (λ') was highest in the 25 °C treatment and lowest in the 19°C treatment (Table 14) and was significantly different among treatments ($F = 92.35$, Cohen's $f = 3.5$, $p < 0.001$) (Figure 12). Differences between the mean λ' of the 19 °C and 31 °C treatments and between the 19 °C and 25 °C treatments contributed to the small p-value, while no detectable difference was seen between the 25 °C and 31 °C treatments (Table 16).

Discussion

Effect of Temperature on Size-Fecundity Relationship

Costanzo et al. (2018) found that the linearity of the size-fecundity relationship varies among developmental temperatures in *Ae. albopictus*. In this study, the linear size-fecundity relationship of *Ae. atropalpus* females that emerged from the lowest temperature treatment had the steepest slope and highest R^2 value, opposite the result seen with *Ae. albopictus* (Costanzo et al. 2018) where the linear relationship weakened with decreasing developmental temperature. However, the slopes of the size-fecundity relationships in this study did not differ significantly among the temperature treatments in this study. Although the temperature ranges used here were non-overlapping, it is possible that the variation in temperature within the treatments weakened the effect on the size-fecundity relationships. Although the slopes did not differ significantly, the goodness of fit of the linear models ranged widely as evidenced by the R^2 values. Costanzo et al. (2018) suggested that it would be more appropriate to develop a size-fecundity relationship by rearing mosquitoes with environmental parameters with close similarity to those that will be used in the experiment instead of taking an existing function from the literature, and that assessment is further supported here.

Effect of Temperature on Finite Population Growth Rate

The hypothesis that the fitness of *Ae. atropalpus* would correlate directly with developmental temperature was partially supported. For both males and females, development was fastest in the 31 ^{o}C treatment, but wing length was also the smallest. The importance of body size for male mosquitoes is not well-understood, although it may influence reproductive success in *Aedes spp*. (Ponlawat and Harrington 2007; De Jesus and Reiskind 2016). Whether the

differences seen here are meaningful to population growth in *Ae. atropalpus* cannot be extrapolated, but the advantage of fast developmental time at 31 ºC is clear. The difference in development time of males and females between the 31 ºC and 25 ºC treatments was much smaller than the difference between the 25 ºC and 19 ºC treatments, implying that development time increases drastically at some colder temperature below the average range of the 25 ºC treatment.

Survival was low for males and females in the 19 ºC treatment, with one major outlier where overall survival was nearly 100%. The outlier was probably a result of the relatively small number of mosquitoes in each experimental unit. Food was provided in excess so that observed mortality could be attributed to environmental temperature instead of resource competition, so it can be assumed that if the surviving mosquitoes from other experimental units had been placed in trays together with bias, that experimental unit would have had high survivorship. The small number of individuals per experimental unit increased the random chance of having an excess of surviving mosquitoes in a single experimental unit. This concern is true for other response variables as well, but their results are more robust because they incorporate more individual data points (e.g. average wing length includes multiple measurements per experimental unit versus one measurement of survival per experimental unit). Even with the outlier, it is apparent that the lower temperature treatment generally resulted in lower survival.

Females had the greatest survivorship, wing length, and fecundity in the 25 ºC treatment, suggesting that their overall fitness was the greatest at that temperature range. However, development time was faster in the 31 ºC treatment, with the average day of eclosion being more than 1.5 days later in the 25 ºC treatment.

Population growth was predicted in each treatment ($\lambda' > 1$), and these high λ' values are likely due to the combination of excess food and low larval density in each experimental unit. Higher larval density and fewer nutritive resources would result in a lower λ' for each treatment, and based on these results, *Ae. atropalpus* exposed to the coldest temperature treatment would be most likely to have their λ' fall below one in a more competitive environment. Lower resource abundance may also exacerbate the difference in λ'between the 25 ºC and 31 ºC treatments. Further studies with varying larval densities and resource treatment should be carried out to gain a more robust understanding of how temperature impacts *Ae. atropalpus* fitness.

Although the treatments are being referred to here as though they were static temperatures, those numbers are actually the grand means of the water temperature of each experimental unit. The temperatures varied under light and dark conditions to create a more realistic scenario (see Methods: Experimental Design). If the overall fitness of *Ae. atropalpus* had been lower in the 25 ºC treatment than the 31 ºC treatment, it could mean that the species is less competitive at the 23 ºC – 27 ºC range, potentially biasing competitive success to *Ae. j. japonicus* at those temperatures, while implying that warmer temperatures where *Ae. j. japonicus* is less abundant are preferable to *Ae. atropalpus* regardless of the presence of the invasive species. Instead, fitness appeared to be somewhat less in the 31 ºC treatment than the 25 ºC treatment, and fitness would presumably suffer at even higher temperatures. This could mean that the optimal temperature for *Ae. atropalpus* larval development is near the optimal developmental temperature of *Ae. j. japonicus*, and that pools with the preferable temperature range for *Ae. atropalpus* are also the pools where competition with *Ae. j. japonicus* is most likely to occur (Byrd et al. 2019). However, the results here clearly imply that *Ae. atropalpus* fitness

suffers at the lower water temperatures observed in Bull Pen rock pools (Aim 1), where *Ae. j. japonicus* was collected year-round but *Ae. atropalpus* was almost never collected.

It is important to recognize that determining the optimal developmental temperature for a mosquito in the laboratory might not be meaningful in the natural world. Specific measures of fitness that are often used in studies of mosquito competition were measured in this study, and although they are useful for analyzing the impacts of environmental differences on larval development, they are unlikely to encapsulate all important factors influencing evolutionary processes on mosquitoes for habitat selection. Under appropriate conditions, autogeny may be advantageous to mosquito populations by allowing females to forego host-seeking and the risks associated with biting (Tsuji et al. 1990). Aquatic habitats with higher temperatures may allow *Ae. atropalpus* to offset the lengthened development times required for acquisition of sufficient nutrient reserves for autogenous reproduction (Armistead et al. 2008). The formula for finite population growth does not consider the risk negation offered by autogenous reproduction. Since other mosquito species are found less commonly in rock pools with high water temperatures (Byrd et al. 2019), avoidance of interspecific competition could be an advantage to larval development at high temperatures. Additionally, the importance of decreased development time may be underweighted for an autogenous species in the finite population growth equation.

Conclusion

In a laboratory experiment with excessive food availability and low larval densities, differences in developmental temperature created significant differences in population growth correlates of the native rock pool mosquito *Aedes atropalpus*. The coldest temperature treatment resulted in lower survival and much longer development times, without significant increases in size or fecundity. The strength of the size-fecundity relationship was greatest at the lowest temperature treatment, providing further evidence of environmentally induced variation in the relationship within mosquito populations. The cold temperature range in the experiment was based on temperatures observed in rock pools at a site where *Ae. atropalpus* is rarely encountered, but where invasive *Ae. j. japonicus* is common. These results suggest that larval development in rock pools with colder temperatures may be detrimental to population growth rates compared to development in warmer pools, even without the presence of *Ae. j. japonicus*. However, population growth estimates trended the highest on average in a mid-range temperature treatment that represented temperatures where both species can be found in comparable abundances in nature, with fitness decreasing slightly at a higher temperature. *Aedes atropalpus* and *Ae. j. japonicus* may have similar optimal development temperatures where competition between the species is the most important.

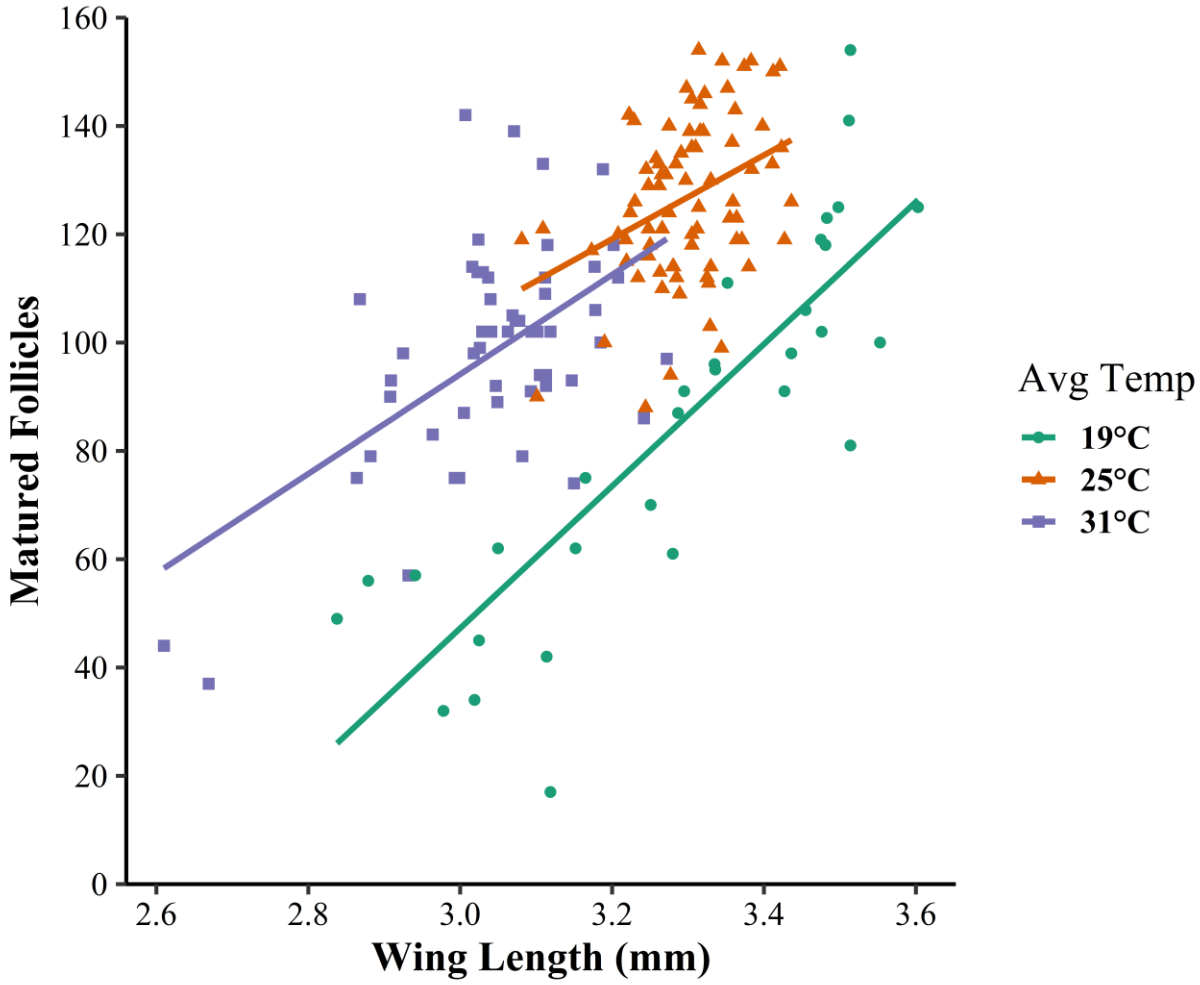

Figure 10. Size-fecundity relationships of *Ae. atropalpus* reared at different temperatures.

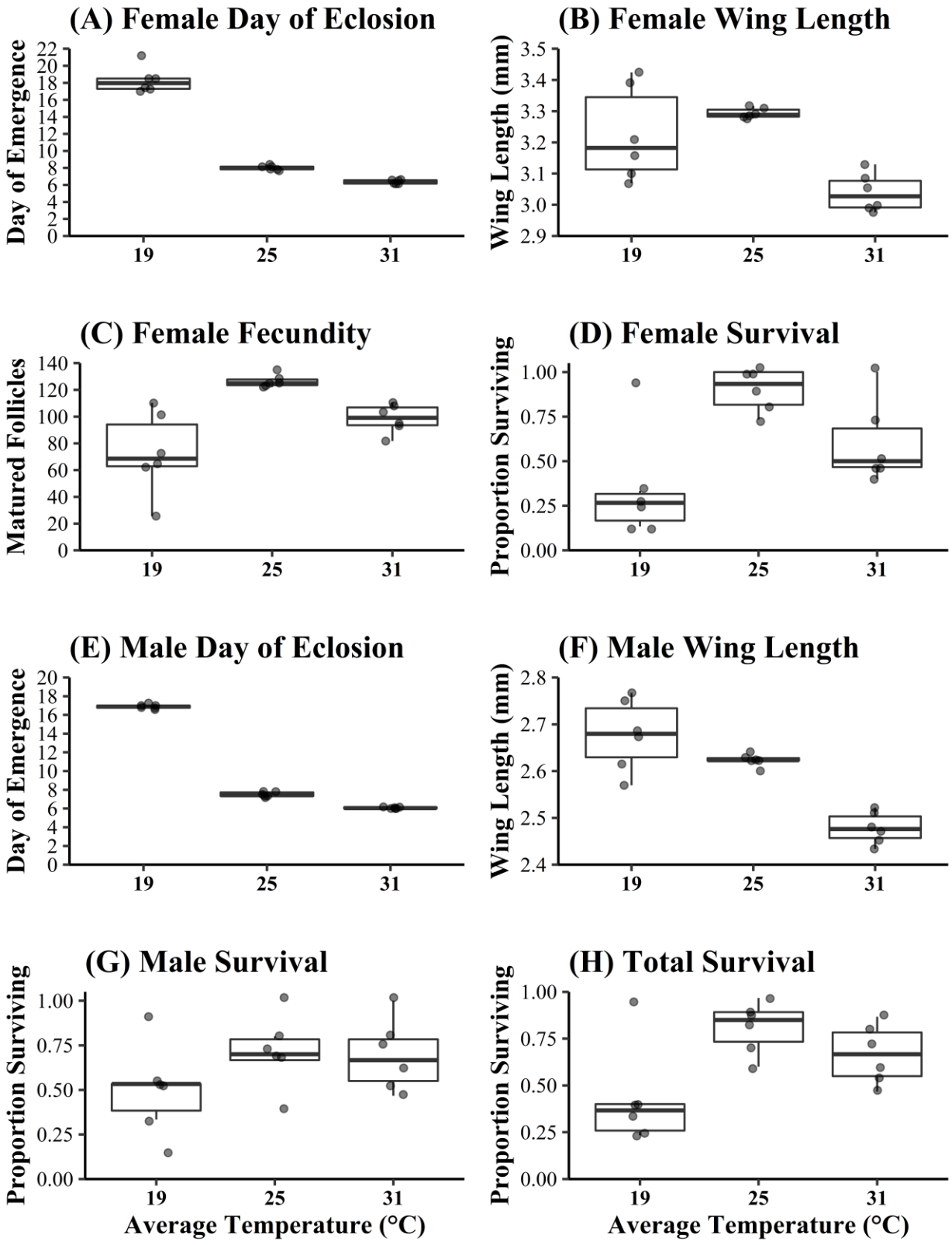

Figure 11. Response variables for *Ae. atropalpus* reared at three temperature ranges.

Table 10. Linear regression of wing length (independent) and fecundity (dependent) and linear equations for functions relating fecundity and wing length (*w*).

Treatment	t (df)	R^2	p	f(w)
All treatments	9.16 (162)	0.34	< 0.001	93.23*w* – 190.03
19 ºC	8.55 (29)	0.71	< 0.001	131.17*w* – 346.26
25ºC	3.52 (78)	0.13	0.001	77.45*w* – 128.73
31 ºC	4.801 (51)	0.30	0.001	91.85*w* – 181.40
25ºC + 31 ºC	12.024 (131)	0.52	< 0.001	103.131*w* – 214.318

Table 11. Summary statistics for males that emerged from the temperature treatments. Values shown are grand means, with ranges based on means of the individual replicates.

Temperature	Day of Eclosion (min – max)	Wing Length (mm) (min – max)	Proportion Surviving (min – max)
19 ºC	16.90 (16.57 – 17.25)	2.67 (2.57 – 2.77)	0.50 (0.13 – 0.93)
25 ºC	7.50 (7.17 – 7.81)	2.62 (2.60 – 2.64)	0.71 (0.40 – 1*)
31 ºC	6.07 (6.00 – 6.17)	2.48 (2.43 – 2.52)	0.69 (0.47 – 1*)

*Two experimental units had more than 15 males emerge; proportions were converted to 1.

Table 12. Tukey's HSD contrasts of MANOVA with male data.

Contrast	Estimated Difference	95% Confidence Interval	p
19 ºC – 31 ºC	10.840	10.23 – 11.45	< 0.001
25 ºC – 31 ºC	1.60	1.03 – 2.16	< 0.001
19 ºC – 31 ºC	-9.242	-9.95 – -8.53	< 0.001

Table 13. p-values of univariate contrasts of MANOVA for males and females with Bonferroni corrections. Each contrast had two degrees of freedom.

	Day of Eclosion	Wing Length	Survival	Fecundity
Males	< 0.001***	< 0.001***	0.987	NA
Females	< 0.001***	< 0.001***	0.016*	< 0.001***

Table 14. Summary statistics for females that emerged from the temperature treatments. Values shown are grand means, with ranges based on means of the individual replicates.

Average Temperature	Day of Eclosion (min – max)	Wing Length (mm) (min – max)	Proportion Surviving (min – max)	Fecundity (min – max)	λ' (min – max)
19 ºC	18.31 (17.0 – 21.2)	3.22 (3.07 – 3.42)	0.34 (0.13 – 0.93)	72.75 (25.5 – 110.25)	1.13 (1.08 – 1.20)
25 ºC	8.01 (7.67 – 8.40)	3.29 (3.28 – 3.32)	0.90 (0.73 – 1*)	126.49 (122.15 – 135.07)	1.37 (1.36 – 1.38)
31 ºC	6.36 (6.13 – 6.63)	3.04 (2.98 – 3.13)	0.60 (0.40 – 1*)	98.60 (81.71 – 110.50)	1.35 (1.31 – 1.41)

*One experimental units had 16 females emerge resulting in a proportion of 1.07 which was converted to 1.

Table 15. Tukey's HSD contrasts of MANOVA with female data.

Contrast	Estimated Difference	95% Confidence Interval	p
19 ºC – 31 ºC	-13.97	-64.79 – 36.86	0.71
25 ºC – 31 ºC	30.091	11.55 – 48.63	0.005
19 ºC – 31 ºC	44.06	-4.43 – 92.55	0.072

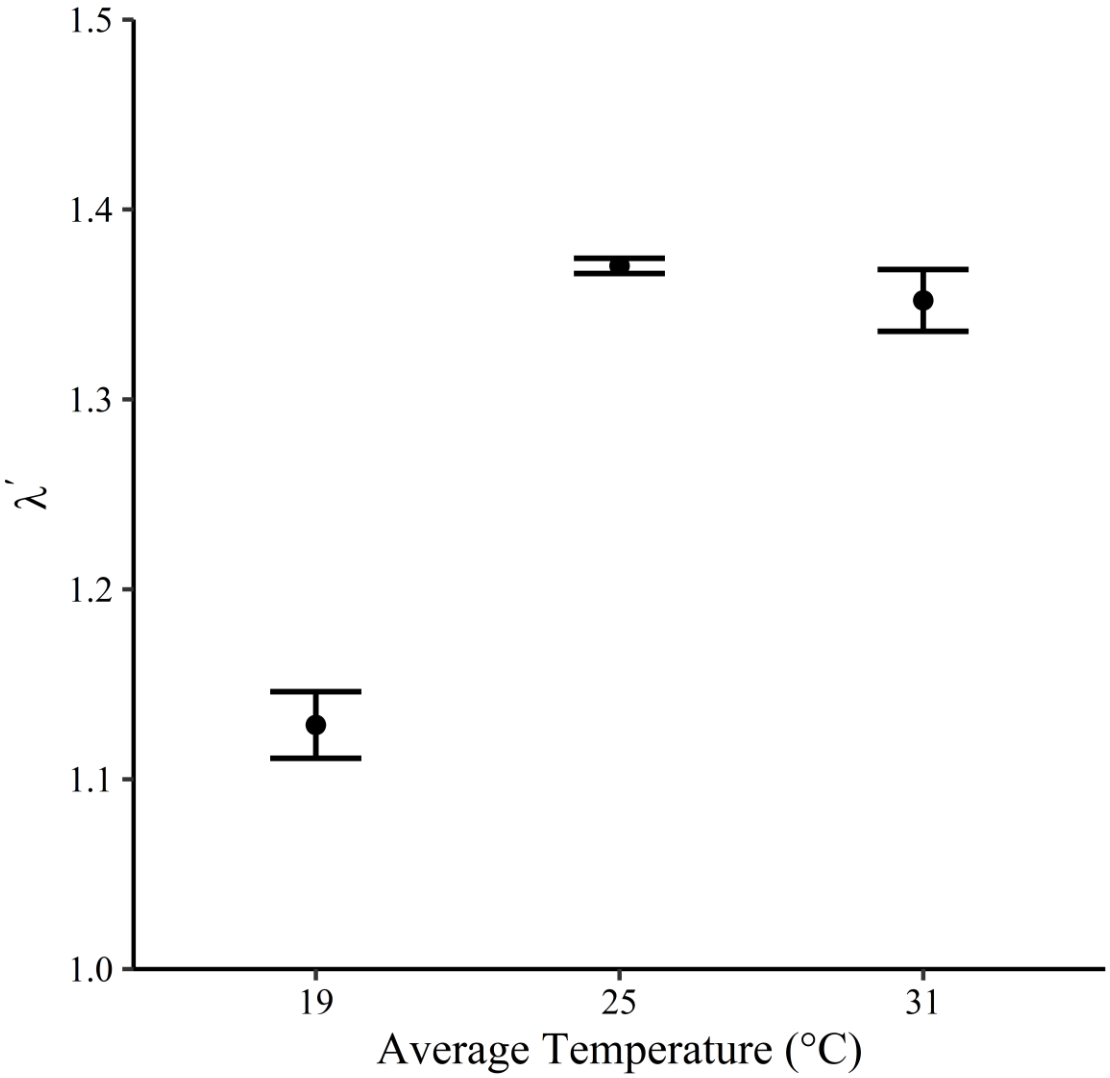

Figure 12. Average finite rate of population growth for *Ae. atropalpus* reared at different temperatures.

Table 16. Tukey's HSD contrasts of average λ' (finite population growth rate).

Contrast	Estimated Difference	95% Confidence Interval	p
19 ^{o}C – 31 ^{o}C	-0.22	-0.28 - -0.17	<0.001
25 ^{o}C – 31 ^{o}C	0.02	-0.03 – 0.07	0.64
19 ^{o}C – 25 ^{o}C	0.24	0.19 – 0.29	<0.001

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